BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
George R. Dubyak	Professor of Physiology and Biophysics
eRA COMMONS USER NAME (credential, e.g., agency login) GDUBYAK	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Saint Josephs University, Philadelphia PA	BS	1974	Biology
University of Pennsylvania, Philadelphia PA	PhD	1979	Cell Physiology
University of Pennsylvania, Philadelphia PA	Post-Doc	1982	Biophysics

A. Personal Statement:

I have been an independent investigator in the field of P2 nucleotide receptor signaling and ion channels as regulators of inflammation and vascular function for 29 years and have authored 126 original research papers and 45 review article/ commentaries/ book chapters. My research in these areas has been funded through R01 and P01 grants from the NIH, as well as grants from the American Heart Association (AHA), including a career development award as an AHA Established Investigator. My laboratory investigates multiple aspects of signal transduction in inflammation, innate immunity, and vascular disease. Three current areas of investigation include: 1) characterization of the NLRP3/caspase-1 inflammasome signaling pathways that mediate local IL-1β-based innate and adaptive immune responses at sites of microbial invasion or host tissue damage and stress; 2) characterization of the mechanisms that differentially direct cells towards apoptosis, necroptosis, or pyroptosis as distinct modes of regulated cell death; 3) the multiple mechanisms by which ATP is released from apoptotic cells or necroptotic cells to act as a chemoattractant for further recruitment of phagocytes to sites of innate immune activation or sterile tissue damage.

B. Positions, Honors, Professional Service, Society Memberships:

1982-1986 Research Assistant Professor of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA.
1986-present Assistant (1986-1990), Associate (with tenure, 1990-1998), and Full (1998-present) Professor of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH.
1987-present Assist./Assoc./Full Prof (Secondary) of General Medical Sciences (Oncology), Assist./Assoc./Full Prof. (Secondary) of Pharmacology, Case Western Reserve University
2004-present Professor (Secondary) of Pathology, Case Western Reserve University
2003-present Co-Director, Medical Scientist Training Program, Case School of Medicine, Case Western Reserve University
1979- 1981 Muscular Dystrophy Association Post-Doctoral Fellow
1989- 1994 Established Investigator of the American Heart Association
2003 Kaiser-Permanente Award for Excellence in Medical School Teaching
2007 Alpha Omega Alpha Medical Honor Society, Elected Faculty Member
2008 Master Teacher, CWRU School of Medicine
1993-1997 NIH Study Section, Cellular Biology and Physiology II (CBY2)
1997-2002 American Heart Association (National) Study Section, Molecular Signaling I
1998-2002 American Heart Association (Ohio Valley Affiliate) Research Committee
1991-2002 Editorial Board, The Journal of Biological Chemistry
2001-2005 Editorial Board, Molecular Endocrinology
2005-2009 Editorial Board, Journal of Immunology
1998-present Editorial Board, American Journal of Physiology (Cell)

2001-present Editorial Board, Molecular Pharmacology
2010-present Editorial Board, The Journal of Biological Chemistry (3rd term)
2010-present Editorial Board, Science Signaling
Member: American Physiological Society; American Society for Biochemistry and Molecular Biology; American Society for Pharmacology and Experimental Therapeutics, American Association of Immunologists, Biophysical Society

C. Contributions to Science (based on 126 original research papers and 45 reviews/ book chapters/commentaries)

1. Characterization of novel innate immune signaling pathways regulated by P2 nucleotide receptors in myeloid leukocytes: My post-doctoral research provided some of the first support for cell surface receptors that are recognized by extracellular ATP/UTP as agonists and coupled to inositol phospholipid hydrolysis and (1,4,5)-$InsP_3$-mediated Ca^{2+} release. Detailed functional characterization of these receptors, eventually defined as the P2Y2 subtype of the larger (8 genetically distinct subtypes) P2Y GPCR family, was the major initial focus of my research as an independent investigator. Those studies determined that P2Y2 and other P2Y subtypes were highly expressed in neutrophils and other myeloid leukocytes. My group subsequently demonstrated that P2Y2 receptors also triggered activation of phospholipase D (PLD) signaling which provides important 2nd messengers for primary granule secretion from neutrophils, a critical early component of the innate immune response. We also surprisingly found that PLD signaling in macrophages could be stimulated by the ionotropic "P2Z" receptor, later identified as the P2X7 subtype of the larger (7 genetically distinct subtypes) P2X family of ATP-gated ion channel receptors. These studies motivated my lab's ongoing work to characterize both the basic cell/molecular biology of the P2X7 receptor and its roles in innate immunity.

a. Cowen DS, Lazarus HM, Shurin SB, Stoll SE, and **Dubyak GR**. (1989) Extracellular ATP activates calcium mobilization in human phagocytic leukocytes and neutrophil/monocyte progenitor cells. *J. Clin. Invest.* 83: 1651-1660. **PMC303873**. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC303873/
b. Xie M, Jacobs LS, and **Dubyak GR**. (1991) Regulation of phospholipase D and primary granule secretion by P_2-purinergic- and chemotactic peptide-receptor agonists in induced during granulocytic differentiation of HL-60 cells. *J. Clin. Invest.* 88: 43-54. **PMC296001**. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC296001/
c. Humphreys BD and **Dubyak GR**. (1996) Induction of the P2Z/ $P2X_7$-nucleotide receptor receptors and associated phospholipase D activity by lipopolysaccharide and γ-interferon in the THP-1 human monocytic cell line. *J. Immunol.* 157: 5627-5637.
d. Verhoef PA, Estacion M, Schilling WP, and **Dubyak GR**. (2003) P2X7 receptor-dependent blebbing and the activation of Rho-effector kinases, caspases, and IL-1β release. *J. Immunol*. 170: 5728-5738. http://www.jimmunol.org/content/170/11/5728.long

2. Characterization of transcriptional and ionic signals that regulate assembly of inflammasome signaling platforms caspase-1 activation, and IL-1β secretion: A major focus of my research for the last 10 years has been to define the mechanisms by which ATP-gated P2X7 receptor channels or other stimuli that perturb ionic homeostasis trigger the very rapid and efficient assembly of NLRP3 inflammasome signaling complexes. The latter mediate accumulation of active caspase-1, caspase-1 processing of IL-1β, and the non-classical secretion of the mature IL-1β from macrophages and dendritic cells. Other studies have identified synergistic roles for both pre-transcriptional (IKK-mediated phosphorylation) and transcriptional (NFκB-based) phases of Toll-like receptor signaling to inflammasome regulation.

a. Kahlenberg JM, Lundberg KC, Kertesy SB, Qu Y, and **Dubyak GR** (2005) Potentiation of caspase-1 activation by the P2X7 receptor is dependent on Toll-like receptor signals and requires NFκB-driven protein synthesis. *J. Immunol.*, 175:7611-7622. http://www.jimmunol.org/cgi/content/full/175/11/7611
b. Martin BN, Wang C, Herjan T, Willette-Brown J, Gulen MF, Zhou H, Bulek K, Franchi L, Sato T, Narla G, Zhong X-P,Alnemri E, Thomas J, Klinman D, Fitzgerald K, Karin M, Nunez G, **Dubyak G**, Hu Y, and Li X (2014) IKKα negatively regulates ASC-dependent inflammasome activation. *Nature-Commun,* 5:4977 doi: 10.1038/ncomms5977 (2014). **PMC42978287**. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4298287/
c. Karmakar M, Katsnelson M, Malak HA, Greene NG, Howell SJ, Hise AG, Camilli A, Kadioglu A, **Dubyak GR**, Pearlman E. (2015) Neutrophil IL-1β processing Induced by pneumolysin is mediated by the NLRP3/ASC inflammasome and caspase-1 activation and Is dependent on K+ efflux. *J Immunol*. 194: 1763-1775. PMC4369676 [Available on 2016-02-15]

d. Katsnelson MA, Rucker LG, Russo HM, **Dubyak GR**. (2015) K^+ efflux agonists induce NLRP3 inflammasome activation independently of Ca^{2+} signaling. *J Immunol*. 194:3937-3952. **PMC4390495** [Available on 2016-04-15]

3. Characterization of inflammasome-mediated pathways for non-classical secretion of IL-1β and other inflammatory mediators: Unlike most inflammatory cytokines, IL-1β lacks a signal sequence for targeting to the canonical ER/Golgi based pathways for constitutive secretion. Our studies have linked non-classical IL-1β secretion to inflammasome-regulated mobilization of exosomes and microvesicles as well as induction of pyroptotic death of macrophages and dendritic cells. These studies have predominantly used P2X7 receptor activation as to initiate the IL-1β processing and release cascade.

a. Qu Y, Franchi L, Nunez G, and **Dubyak GR** (2007) Non-classical IL-1β secretion stimulated by P2X7 receptors is dependent on inflammasome activation and correlated with exosome release in murine macrophages. *J. Immunol.* 179: 1913-1925. http://www.jimmunol.org/cgi/content/full/179/3/1913

b. Qu Y, Ramachandra L, Franchi L, Mohr S, Harding CV, Nunez G, and **Dubyak GR** (2009) P2X7 receptor-stimulated secretion of MHC-II-containing exosomes requires the ASC/NLRP3 inflammasome but is independent of caspase-1. *J. Immunol*. 182:5052-5062. **PMC2768485.** http://www.jimmunol.org/cgi/content/full/182/8/5052

c. Ramachandran L, Qu Y, Wing Y, Lewis CJ, Cobb BA, Boom WH, **Dubyak GR**, and Harding CV (2010) Mycobacterium tuberculosis synergizes with ATP to induce release of microsomes and exosomes containing MHC-II molecules capable of antigen presentation. *Infection and Immunity*, 78: 5116-5125. PMC2981298 http://iai.asm.org/cgi/content/full/78/12/5116?view=long&pmid=20837713

d. **Dubyak GR** (2012). P2X7 receptor regulation of non-classical secretion from immune effector cells. (Invited review). *Cell. Microbiol*. 14: 1697-1706. **PMC3473166** http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3473166/

4. Cellular mechanisms for extracellular accumulation and metabolism of ATP and other nucleotides in inflammation, apoptosis, and necroptosis: A parallel component of my group's focus on signaling by extracellular ATP has been characterization of the mechanisms by which ATP is exported into, and metabolized within, extracellular compartments. We established novel methods involving cell surface-immobilized luciferase to track highly localized release and clearance of extracellular ATP in several cell models, including astrocytes, macrophages, T cells, and tumor cells . Other studies have defined specific roles of various ecto-nucleotide phosphohydrolases or pyrophosphatases in the conversion of extracellular ATP to other bioactive mediators (e.g. adenosine or pyrophosphate) in different cell or tissue models. Recent studies have focused on defining how plasma membrane pannexin-1 channels are regulated to function as a major conductive pathway for ATP efflux from apoptotic cells and possibly in other modes of regulated cell death.

a. Joseph SM, Buchakjian MR, and **Dubyak GR**. (2003) Colocalization of ATP release sites and ecto-ATPase activity at the extracellular surface of human astrocytes. *J. Biol. Chem*. 278: 23331-23342. http://www.jbc.org/content/278/26/23331.long

b. Blum AE, Walsh BC, and **Dubyak GR** (2010) Extracellular osmolarity modulates G protein-coupled receptor dependent ATP release from 1321N1 astrocytes. *Am J. Physiol. Cell*. 298: 386-396. **PMC2822496** http://ajpcell.physiology.org/content/298/2/C386.long

c. Qu Y, Misaghi S, Newton K, Gilmour LL, Louie S, Cupp JE, **Dubyak GR**, Hackos D, and Dixit VM (2011). Pannexin-1 is required for ATP release during apoptosis but not inflammasome activation. *J.Immunol.* 186: 6553-6561. http://www.jimmunol.org/content/186/11/6553.long

d. Boyd-Tressler A, Peneula S, Laird DW, and **Dubyak GR**. (2014). Chemotherapeutic drugs induce ATP release via caspase-gated pannexin-1 channels and a caspase/pannexin-1 independent mechanism. *J Biol Chem*. 289:27246-27263 **PMC4175357** [Available on 2015/9/26]

5. Characterization of regulated cell death signaling pathways during innate immune response, tissue damage, and metabolic stress: Our ongoing studies of proinflammatory signaling and ATP release mechanisms have also directed additional research into how these responses are integrated with the various modes of regulated cell death. For example, we determined that brief activation of P2X7 receptors in the absence of toll-like receptor signaling or inflammasome signaling triggers apoptotic death of macrophages. In contrast, activation of the same receptors in inflammatory macrophages or microglia elicits caspase-1-mediated pyroptotic death. Delineation of the molecular mechanisms by which caspase-1 triggers pyroptosis, an intrinsically proinflammatory mode of regulated lytic cell death, is currently a major area of investigation. .

a. Humphreys BD, Rice J, Kertesy SB, and **Dubyak GR**. (2000) SAPK/JNK activation and apoptotic induction by the macrophage P2X7 nucleotide receptor. *J. Biol. Chem*. 275: 26792-26798. http://www.jbc.org/content/early/2000/06/14/jbc.M002770200.long

b. Verhoef PA, Kertesy SB, Lundberg KC, Kahlenberg JM, and **Dubyak GR**.(2005) Inhibitory effects of chloride on the

activation of caspase-1, IL-1β secretion, and cytolysis by the P2X7 receptor. *J. Immunol.*, 175:7623-7634. http://www.jimmunol.org/content/175/11/7623.long

c. Qiu J, Tsien C, Thapalaya S, Narayanan A, Weihl CC, Ching JK, Eghtesad B, Singh K, Hazen SL, **Dubyak G**, McDonald C, Almasan A, Prasad SVN,and Dasarathy S. 2012. Hyperammonemia-mediated autophagy in skeletal muscle contributes to sarcopenia of cirrhosis. *Am J. Physiol. Endocrinol Metab*. 303: E983-993. **PMC3469607** http://ajpendo.physiology.org/content/303/8/E983.long

d. Antonopoulos C, El-Sanadi C, Kaiser WJ, Mocarski ES, and **Dubyak GR**. (2013) Pro-apoptotic chemotherapeutic drugs induce non-canonical processing and release of IL-1β via caspase-8 in dendritic cells. *J. Immunol*. 191:4789-4803. **PMC3870469** http://www.jimmunol.org/content/191/9/4789.long

D. Research Support

NIH R01-GM36387 (22-25) (PI: G. Dubyak) 03/01/11 - 02/28/15 (Extension through 02/28/16)
"Regulation of Caspase-1 Signaling and Inflammation by the P2X7 ATP Receptor"
G. Dubyak Role: PI
Goals: The major goals: are: 1) to characterize the molecular mechanisms by which inflammasome signaling and IL-1β production is activated by extracellular ATP-dependent and extracellular ATP-independent mechanisms in dendritic cells to regulate immunogenic anti-tumor responses: 2) to define the cellular mechanisms for regulated release of ATP from apoptotic tumor cells.

NMSS RG 5130A2/1 (PI: X. Li) 10/01/14 – 09/30/17
"Cellular and molecular mechanisms of the inflammasome in CNS inflammation"
National Multiple Sclerosis Society
G. Dubyak Role: Co-investigator
Goals: The major goal is to characterize canonical and non-canonical mechanisms of inflammasome activation in antigen-presenting cells and T cells in mouse models of demyelinating diseases.

5R01-EY014362 (10-14) (PI: E. Pearlman) 01/01/14 – 12/31/18
"Innate Immunity in Bacterial Keratitis"
G. Dubyak Role: Co-investigator
National Institutes of Health/ National Eye Institute
Goals: The project seeks to define the role of the inflammatory cytokine IL-1beta in regulating corneal disease due to either Staphylococcus aureus (MRSA) or Streptococcus pneumoniae infection. One aim led by the Dubyak lab examines the role of extracellular ATP and host cell purinergic receptors in amplifying inflammasome activity and IL-1beta production during bacterial keratitis in the cornea.

AHA 13PRE16860052 (M. Katsnelson) 07/01/2013-06/30/2015
"Regulation of NLRP3 Inflammasome Activation and IL-1β Release by Loss of Lysosomal Integrity"
American Heart Association Pre-Doctoral Fellowship
G. Dubyak Role: Fellowship Sponsor and Dissertation Research Mentor
Goals: The major goal of this fellowship is to characterize how perturbation of plasma membrane and organellar ion homeostasis mediates the activation of inflammasomes and IL-1β secretion by proinflammatory crystals and amyloid aggregrates that disrupt lysosome integrity.

NIH T32-GM007250-38 (PI/PD: C.V. Harding) 7/1/2014-6/30/2019
"Medical Scientist Training Program"
G. Dubyak Role: Co-Director
This is a training grant for pre-doctoral MD, PhD candidates.

NIH T32-HL105338-04 (PI/PD: M. Jain) 09/20/2010-08/31/2015
"Cardiovascular Research Training Program –CRTP"
G. Dubyak Role: Co-Director
This is training grant for pre- and post-doctoral trainees in cardiovascular-related research.